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Review

Redox-Modulation of Chloroplast Enzymes[1]

A Common Principle for Individual Control

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ABSTRACT

Assimilation of C, N, and S into organic compounds requires effective and flexible cooperation among the energy-converting, tightly coupled, thylakoid-bound processes and stromal metabolism. Fluctuations of light, temperature, and changing concentrations of the various reducible substrates pose unique regulatory problems to photoautotrophic plant cells. Covalent redox modification of enzyme proteins as mediated by the ferredoxin/thioredoxin-system is suited to provide short-term adaptation of various enzymatic activities in the chloroplast. This mode of regulation is based on the continuous turnover of interconvertible enzyme forms, as in the systems driven by protein phosphorylation/dephosphorylation, but is particularly adapted to the unique conditions of a compartment performing oxygenic photosynthesis by depending on the simultaneous presence of reducing power and of oxygen. Individual fine control of each of the enzymes subjected to redox modification is achieved by specific metabolites acting as additional positive or negative effectors of the reductive (and/or oxidative) modification reaction. The biochemical prerequisite for such a control is the presence of regulatory (extra) sequences carrying cysteine residues which are subjected to reversible redox changes. Although no common amino acid sequence has yet been identified among the known regulatory peptides, in all cases the evolution of autotrophy should be related to the presence of extrasequences in otherwise very conserved enzyme molecules.

Light activation of chloroplast enzymes is a process linked to photosynthetic electron transport via the ferredoxin/thioredoxin system. The activities of the target enzymes change within minutes upon illumination or darkening: the Calvin-cycle enzymes fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, and phosphoribulokinase, the "malate valve" enzyme (16) NADP-malate dehydrogenase, and the chloroplast coupling factor CF_1 (14) are switched on upon illumination (1, 2), while chloroplast glucose-6-P dehydrogenase becomes active in the dark (18). This system has been described recently in detail (1, 2, 8, 9, 16, 17). Inasmuch as the enzymes under discussion belong to pathways which should either function in the light or in the dark (*e.g.* reductive and oxidative pentose phosphate cycle), the purpose of that kind of regulation was thought to be in place to avoid futile cycles. Thus, light/dark redox modulation was considered predominantly as a means to provide an on/off switch for certain key enzymes. However, more recently it became evident that such a system also provides a means to strictly control enzyme activities in the light.

REGULATION IN THE LIGHT

In addition to requirements arising from the change between "light" and "dark" metabolism, also problems caused by varying conditions during the light phase have to be met by control mechanisms of the chloroplast. Depending on the flux of CO_2, the available light intensity, etc., on the one hand and the demand for carbohydrates and other organic compounds on the other, the velocities of the various steps need to be carefully adjusted. Generally, such metabolic control is accomplished by interconvertible enzyme forms subjected to covalent modification, *e.g.* phosphorylation/dephosphorylation. The mode of modification which is adjusted to the situation in the chloroplast is the reduction/oxidation of cysteine residues. The thioredoxin-mediated reduction process requires photosynthetic electron transport. Oxygen, evolved in the water splitting reaction, causes continuous reoxidation of the regulatory thiol groups of thioredoxin and, as a consequence, of the target polypeptide during photosynthesis. At steady state, the relative rates of reduction and reoxidation will determine the amount of active enzyme.

Because various metabolites influence the rate constants of reduction or oxidation (17), probably by changing the midpoint potentials of the regulatory sulfhydryls, the ratio of reduced to oxidized enzyme appears to be effectively adjusted to changes in metabolism responding to changing pool sizes of specific metabolites. As an example, an increasing fructose-1,6-bisP level acts as positive effector for fructose-1,6-bisphosphatase activation (reduction), thus catalyzing an increased turnover of fixed carbon inside the chloroplast (into starch and into the CO_2-acceptor ribulose-1,5-bisP). On the other hand, high NADP levels are inhibitory for NADP-malate dehydrogenase activation. This appears to limit export of reducing equivalents via malate (*versus* oxaloacetate), when sufficient reoxidation of NADPH is accomplished by reductive processes inside the chloroplast (see below). In conclusion,

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by means of such individual control of the various regulated steps which are subject to reduction/oxidation, it is possible to envision a tuning of the various portions of the primary assimilation pathways individually, while still adjusting the overall fluxes to the required demand.

LIGHT/DARK MODULATION OF NADP-MALATE DEHYDROGENASE AS CONTROL OF THE "MALATE VALVE" OF C_3-CHLOROPLASTS

In principle, the light-driven generation of NADPH and of ATP are strictly coupled reactions as a consequence of the linear electron flow. On the other hand, C, N, and S assimilation into various organic compounds requires flexibility of the ATP:NADPH ratio depending on the supply of the substrates (environmental conditions) and on the demand for the various products (metabolic and developmental state of the plant). The ATP:NADPH ratio required for the synthesis of carbohydrates is probably higher than that produced upon linear electron transport. Since the plant cannot escape the local situation and must make the maximal use out of it, a strategy has been developed for an optimal adjustment of the chloroplast energy production and utilization. Frequently, the capacity of electron flow is in excess of that required to meet the required rate of ATP synthesis. Such an imbalance would result in an overreduction, which would in turn feed back on electron flow. A means for poising NADPH and ATP production to their rates of consumption is the export of excess reducing equivalents which is achieved by the malate valve (16).

The valve-type mechanism could be demonstrated in action by responding with oscillations induced by sudden changes of experimental conditions, *e.g.* of the light intensity (19). Even under steady-state conditions (under saturating CO_2) about one-third of the total capacity of 100 to 200 μmol reducing equivalents per mg Chl·h appears to be active (19). It is tempting to speculate that malate, exported from the chloroplast, might supply the mitochondria of green cells with reducing equivalents which in turn could lead to ATP production. ATP could reenter the cytosol serving metabolic requirements, *e.g.* biosynthesis of sucrose. In the dark, when there is no need for that kind of poising, turning off the "main switch" via oxidation will prevent any export of reducing equivalents.

PROPERTIES OF THE CHLOROPLAST REDOX REGULATORY SYSTEM

First, some kinetic and energetic considerations deduced from various observations that have been made for the chloroplast system will be presented, although detailed systematic studies for each of the enzymes are largely lacking. *In vivo,* the activities of the chloroplast target enzymes fructose-1,6-bisphosphatase and NADP-malate dehydrogenase are found to be determined by the required fluxes (11, 17). Thus, changes of a signal metabolite (*e.g.* NADP-concentration) result only in a relatively small change of enzyme activity. This is in contrast to the ultrasensitive animal systems that exhibit a "switch or threshold" behavior responding exclusively to a certain "threshold value" (10). The advantage of the chloroplast system appears to be its linear responsiveness over a wide range of metabolic and environmental conditions as encountered by a photoautotrophic organism.

As for each regulatory system, also in the case of the chloroplast the question of the energy cost has to be considered. The thioredoxin-mediated, light-activation system consumes photosynthetic reducing power, while the protein-kinase/protein-phosphatase system requires energy provided by ATP (6). In the former case, it is expected that ATP will be produced even when electron flow is to target enzymes, but the total flux is rather small. The rate of electron flow to the target enzyme is a function of the turnover time of the interconvertible enzyme forms (oxidized and reduced form) that is supposedly in the minute range. Apparently there is no need for a multistep cascade system, since the environmental conditions encountered by a plant in most cases change gradually rather than abruptly. The delay of the response to sudden (laboratory generated) changes can be seen as oscillations with a 1- to 2-min dampening phase leading to altered homeostatic levels (19).

Thioredoxin-catalyzed reactions have been described in numerous animal and bacterial systems (7) and the possibility of reversible thiol-disulfide exchange as a means for regulation of metabolism has been suggested (20). However, in none of those nonplant systems has a true correlation between changes of the redox state and the activity yet been established *in vivo* (5). Thus, in the chloroplast, the simultaneous presence of oxygen and of reducing power appears to create the unique conditions necessary for the redox modification of enzymes as a means of short-term regulation. However, for some plant cytosolic enzymes, as well as for various other chloroplast enzymes which have been suggested in the past to be "light-modulated," the above mentioned evidence for reversible redox modification is still lacking.

Finally, the primary structures of the enzymes in question will be considered in a comparative way. For all of the redox-regulated chloroplast enzymes, counterparts that catalyze the same reaction but occur in other compartments or organisms are known. However, the activities of these enzymes are either subjected to another, noncovalent type of control or are not adjustable at all. Now that many sequences are known, the "regulatory peptides" containing the cysteine residues involved in redox modulation of most of the chloroplast enzymes have been identified and are found to be different for the different enzymes. That is, there is no common feature detectable in the regulatory peptides of fructose-1,6-bisphosphatase (12), phosphoribulokinase (15), NADP-malate dehydrogenase from pea (4) or corn (3), and of the γ-subunit of ATPase (13), either with respect to sequence homology or to their location within the whole polypeptide (see ref. 9). Interestingly, the partial removal of the extrapeptides of chloroplast NADP-malate dehydrogenase results in well-defined enzyme forms with changed properties (17). The study of partially proteolized forms as well as the search for intermediate or possible precursor forms, tracing evolutionary development of the redox-modulation system, is of particular fascination.

CONCLUSIONS

1. Light-dark modulation of the catalytic capability of chloroplast enzymes is achieved by interconversion of two

forms of one enzyme: the reduced (dithiol) and the oxidized (disulfide) form. The redox modulation is mediated by the ferredoxin/thioredoxin system, which is reduced by the photosynthetic electron transport and reoxidized by the concomitantly produced oxygen. Close interaction with the target enzymes transmits the redox status of the ferredoxin/thioredoxin system to these proteins. Hence, in the light, the target enzyme is subjected to a continuously running oxidation-reduction cycle, while, in the dark, the turnover stops and the ratio of reduced to oxidized enzyme is completely shifted towards the oxidized form.

2. Running continuously through a reduction-oxidation cycle necessitates another system of control in order to adapt the ratio of reduced to oxidized form and thereby the activity of the enzymes to the actual requirements of the chloroplast. Such fine-tuning is brought about by the interference of effectors with the rate of reduction.

3. Simultaneously, this mechanism allows the independent adjustment of various enzyme activities. Special signal metabolites characteristic for the metabolic status at each of the various key steps control the fluxes through the respective parts of the pathway. Such metabolites are, for example, fructose-1,6-bisP for fructose-1,6-bisphosphatase and NADP for NADP-malate dehydrogenase. Both enzymes accomplish completely different physiological functions in the chloroplast.

4. The redox-modulated enzymes are furnished with unique regulatory peptides added at different positions to the otherwise conserved amino acid sequences. These extra peptides contain the regulatory dithiol/disulfide.

5. The expense for that type of regulation is the investment of a comparably small portion of the photosynthetic reduction power to drive the reduction-oxidation wheel, comparable to the ATP expenditure for driving protein phosphorylation/dephosphorylation for similar regulatory purposes in other systems.

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